Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Synthesis of Hollow Mesoporous Ruthenium Nanoparticle: Evaluation of Physico-Chemical Properties and Toxicity

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Section S1

Study of cell behavior and toxicity using ECIS method

HEMa and B16 F10 cells were used in this study, which is cultured under standard protocol. ECIS system was used to measure the changes of cellular behavior and toxicity upon adding NPs for real-time monitor, as formerly reported by Keese and Giaever.[1] This study 8W10E array was used, which consist of eight wells each containing ten gold detecting electrode (250 μm size) and the counter electrode. The circuitry connections of all microelectrodes are connected to the array edge, and were connected to the lock-in amplifier.[2] The electrode containing well was washed with PBS buffer, then added with 250 μL of fresh media, and the setup was kept in an incubator and maintained at 37°C and 5 % CO_2 for 30 min to get background impedance value (Z_0). The data acquisition and processing were performed using the Applied Biophysics software. At the same time, HEMa and B16 F10 cells were prepared and exact concentration of (5×10^5) cells was added to the wells and all wells were adjusted to the level of 400 μL by using respective growth media. After the attachment of cells, freshly prepared medium containing different concentration (50 -500 μg ml^{-1}) of F1 were added by substituting the initial medium, and the cellular behavior and toxicity were continuously monitored for 24 hr. Control (without drug treatments) also maintained in each experiment to compare the cellular behavior and toxicity.

Cellular uptake study

Cellular uptake of hollow mesoporous Ru NPs by HEMa and B16 F10 cells were studied by fluorescence microscope.[3] Cells were taken after 48 hr subconfluent, and cultured in six-well plates and incubated at 37°C and 5 % CO_2 for 10 hr. After cell attachment, the freshly prepared

medium containing a lowest toxic concentration (100 μg ml^{-1}) of F1 were added into the wells and were incubated for 12 hr. Control (without NPs added) also maintained in each experiment and both cells were compared to cell uptake. After 12 hr, cells were washed with PBS to remove unbounded NPs. Then the cell nucleolus was stained with Alexa Fluor 488 and DAPI by following the standard protocol and imaged under a fluorescence microscope, and processed by the ImageJ software (ImageJ, National Institutes of Health (NIH), Bethesda, MD). Images (including z-stacks) of the NPs internalized and attachment on the surface were observed using a confocal microscope (Zeiss LSM 700; Carl Zeiss Micro-Imaging GmbH, Germany). Above all experiments were carried out in triplicate, the data were compared with the corresponding control experiments and the data presented are the average standard values.

Loading of DOX onto hollow mesoporous Ru NPs and release

5 mg of hollow mesoporous Ru NPs was added to the 1 mL of phosphate buffer solution containing 2.5 mg of DOX. The concentrated mixture was sonicated for 1 hr in ultrasonic cleaner (50 Hz) at 30˚C and stirred in the dark for 24 hr in magnetic stirrer at constant speed to increase the dispersion of the NPs. After the dispersion, DOX-loaded hollow mesoporous Ru NPs were collected by centrifugation and the sediment was washed with PBS buffer solution. The amount of DOX loaded into hollow mesoporous Ru NPs was calculated by subtracting the mass of DOX present in the supernatant from the initial total mass of DOX in the loading solution using UV-visible spectroscopy.

To determine the drug release from these NPs, the 5 mg of DOX-loaded hollow mesoporous Ru NPs were dispersed in 5 mL of PBS (0.1 M, pH 7.4 and pH 5.0) in a dialysis membrane bag (MWCO: 3500 Da, Spectra/Por® Biotech Cellulose Ester Dialysis Membranes),

and the bag was dip in 50 mL of PBS at 37˚C under constant gentle stirring. At pre-determined time intervals, 1 mL of release medium was taken and analyzed the amount of DOX released at over a period of 48 hr using UV-visible spectroscopy at 480 nm, and equivalent volume of fresh PBS was replaced. The released DOX were estimated, and the percentages of DOX released from the hollow mesoporous Ru NPs were plotted against time. The studies were performed in triplicate. SD was calculated by origin pro 7.5 plots.

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Table S1.

Ingredients	Formulation code		
	F1	**F2**	**F3**
$RuCl_3$ (precursor)	20 mM	20 mM	20 mM
Perchloric acid	0.2 M	0.1 M	0.05 M
Poloxamer 407	50 mg	75 mg	100 mg
NH-Si nanoparticles (100 nm)	3 mg	3 mg	3 mg
$NaBH_4$	100 mM	100mM	100mM
Hydrofluoric acid	20%	20%	20%
Physico-chemical characterization parameters			
Size (nm)	162 ±6.0	183±9.7	244±14.0
PDI	0.17±0.05	0.24±0.06	0.41±0.03
Zeta potential (mV)	-22.06±1.5	-20.8±1.5	-17.3±1.7

Table 1. Compositions of various formulations of hollow mesoporous Ru NPs and its characterizations. Each value represents the mean ± SD (n=3).

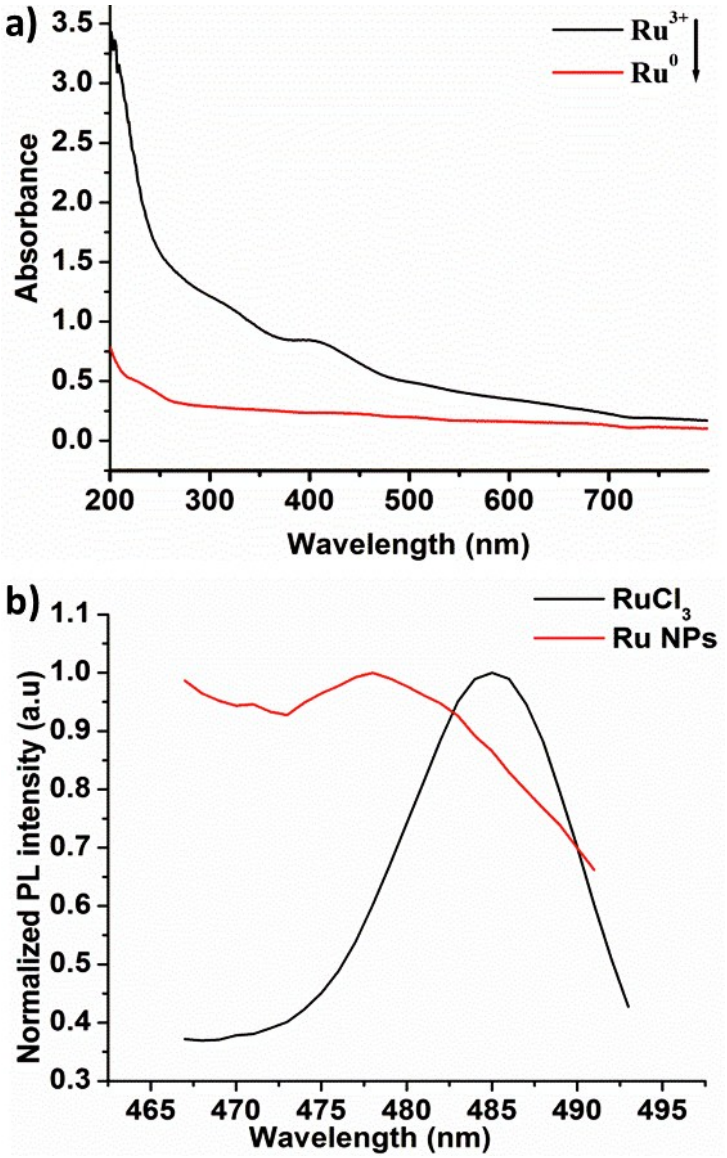

Fig. S1: (a), UV-visible absorption spectra of the ruthenium nanoparticle reduction from Ru^{3+} to Ru^{0} state. (b), Fluorescence emission spectra of the ruthenium nanoparticle (pre-synthesized and synthesized).

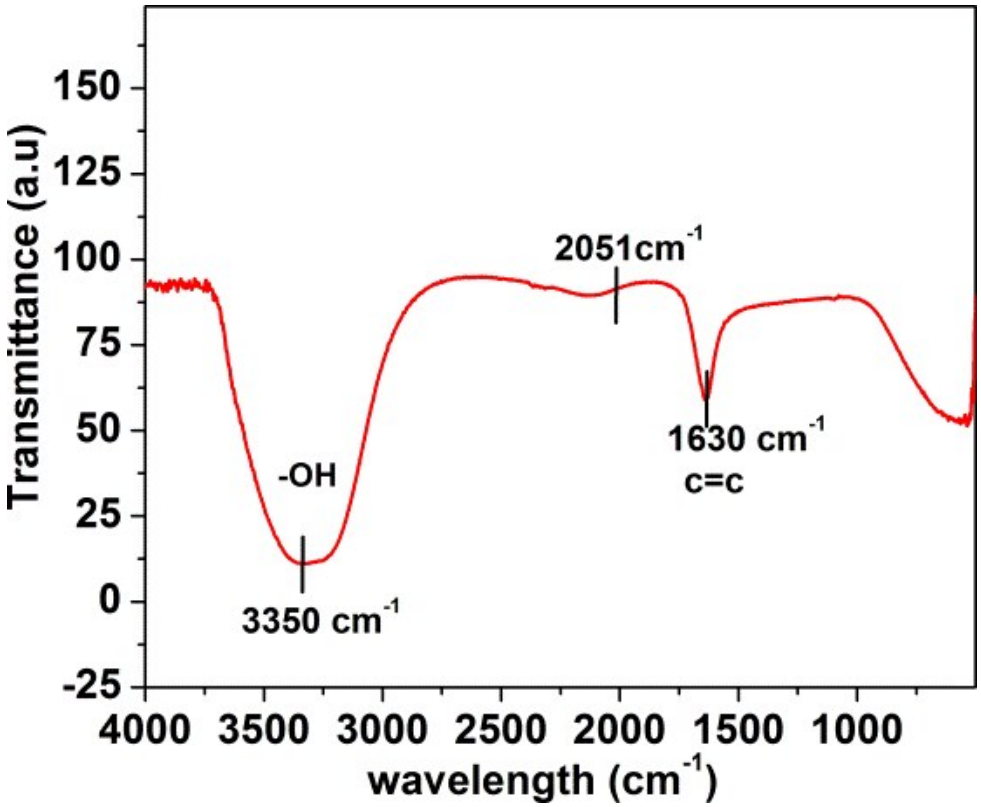

Fig. S2: FT-IR spectral functional group evaluation of synthesized hollow mesoporous Ru NPs

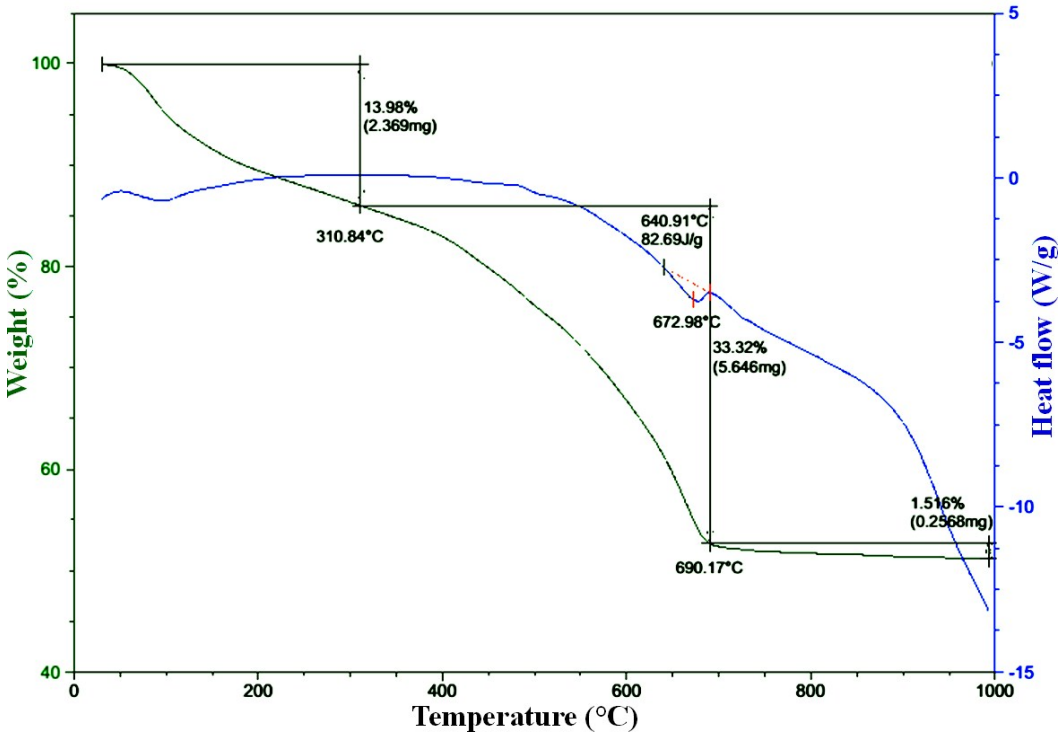

Fig. S3: TGA and DSC curve of hollow mesoporous ruthenium nanoparticle.

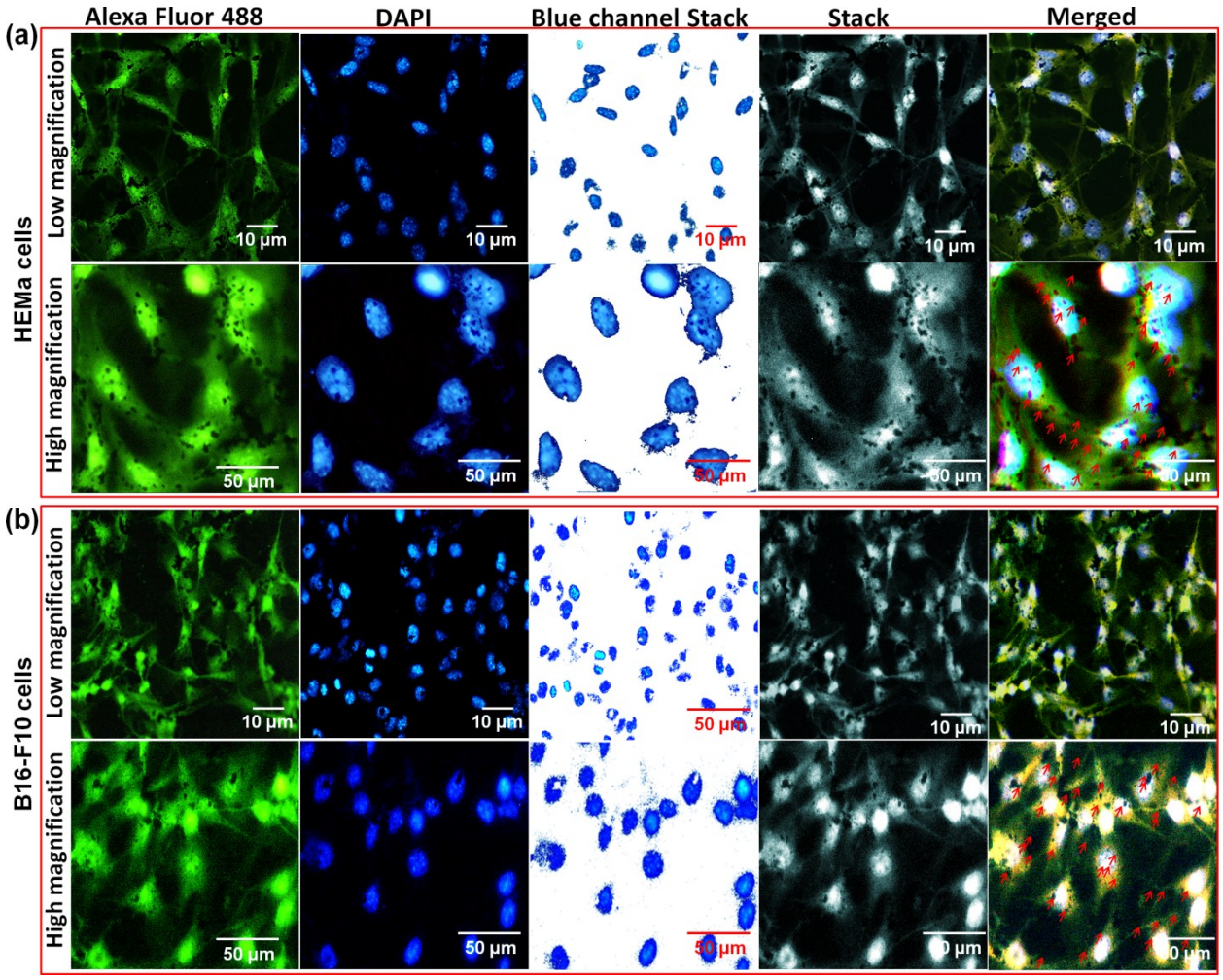

Fig. S4: Fluorescence microscopy images with different magnifications for cellular uptake of hollow mesoporous Ru NPs. Stained with microtubules (green, Alexa Fluor 488), nuclei (blue, DAPI), respectively. a) HEMa cell image with lower magnification in upper panel and higher magnification in lower panel. b) B16-F10 cell image with lower magnification in upper panel and higher magnification in lower panel.

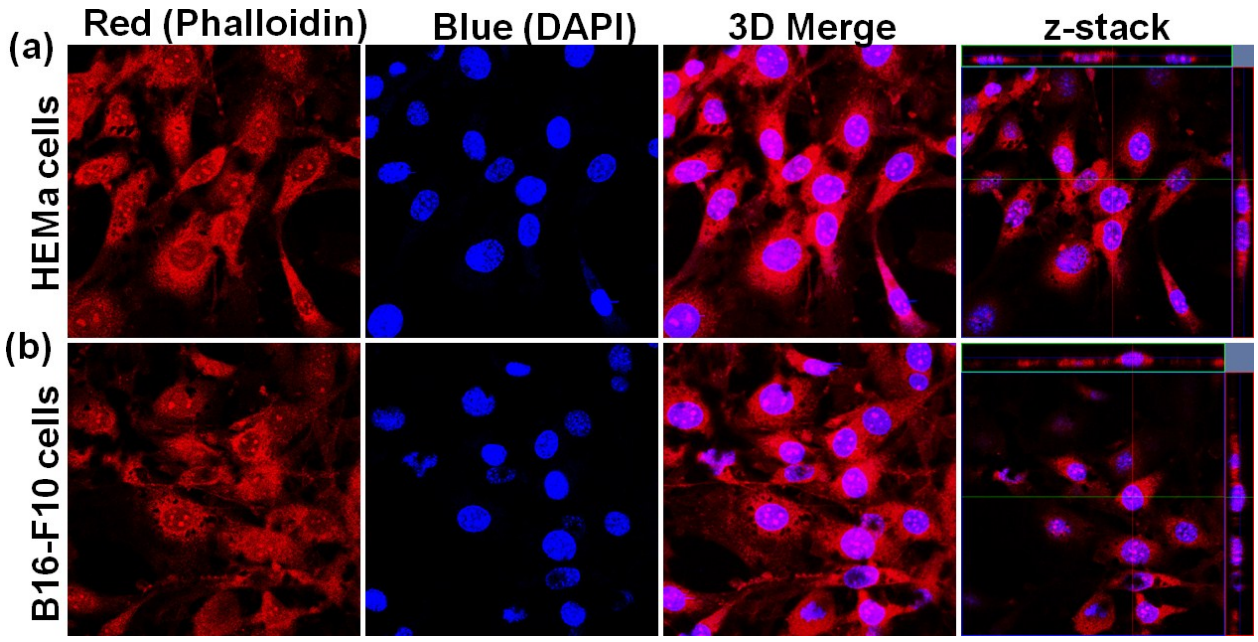

Fig. S5: Confocal microscopic analysis (including z-stacks) of NPs uptake by the cells. The NPs were internalized and attached on the surface of both cell lines. (a) HEMa cell images were show in upper panel. (b), B16-F10 cell image show in lower panel.

REFERENCES

1. I. Giaever and C. R. Keese, *Nature*, 1993, **366**, 591-592.
2. S. Ramasamy, D. Bennet and S. Kim, *Int. J. Nanomedicine*, 2014, **9**, 5789-5809.
3. D. Bennet, S. C. Kang, J. Gang and S. Kim, *Int. J. Nanomedicine*, 2014, **9**, 93-108.